

one swab was processed using the optimized experimental protocol and the second swab was processed with a validated standard differential extraction protocol used by Broward Sheriff's Office (BSO) crime lab. A comparative study was done to evaluate the ability of this method to separate mixtures in sexual assault samples. The ratio of autosomal to Y-chromosomal DNA concentrations was calculated using Plexor® HY software for epithelial and sperm fractions. A value closer to 1.0 indicates the ability to obtain a predominantly male autosomal STR profile whereas a higher value indicates the presence of more female alleles.

TABLE 2

A comparison of DNA recoveries and STR profiles of post-coital samples obtained with a validated extraction protocol to the results obtained with alkaline lysis and pressure cycling technology.				
	Volunteer # 1	Volunteer # 2	Volunteer # 3	
Time lapse since intercourse	0-5 hrs	7-12 hrs	13-18 hrs	
BSO <sup>a</sup> method				
[Auto]/[Y] in epithelial fraction <sup>c</sup>	2.9	38	27	
[Auto]/[Y] in sperm fraction <sup>c</sup>	0.5	0.8	1.0	
Alkaline lysis with pressure cycling <sup>b</sup>				
[Auto]/[Y] in epithelial fraction <sup>c</sup>	3.4	30	59.2	
[Auto]/[Y] in sperm fraction <sup>c</sup>	0.6	46.4	2.4	
Ratio of X/Y alleles at Amelogenin locus: PP16 HS <sup>d</sup> (BSO method)	1:1 [M]	1:1 [M]	1:1 [M]	
Ratio of X/Y alleles at Amelogenin locus: PP16 HS <sup>d</sup> (Alkaline lysis + pressure cycling)	1:1 [M]	No Male DNA Detected [F]	5.4:1 [Mixture]	

<sup>a</sup>Differential extraction protocol used by Broward Sheriff's Office (BSO) crime lab is used for comparison with the current protocol in development.

<sup>b</sup>Alkaline lysis was used in conjunction with pressure cycling technology (PCT) to determine the ability of this method to analyze rape kits.

<sup>c</sup>Plexor® HY system (Promega) was used to determine the ratio of autosomal to male (Y) DNA.

<sup>d</sup>PowerPlex® 16 HS system (Promega) was used to generate DNA profile (M = male DNA profile; M + F = mixed DNA profile; major = major component of the mixed DNA profile).

The results from alkaline lysis and pressure cycling of post-coital swabs obtained from volunteer #2 and volunteer #3 showed that when the ratio of autosomal to Y-chromosomal DNA concentrations in epithelial fraction exceeded 30, indicating an excess of female tissue, and the incomplete digestion of vaginal epithelial cells led to female DNA carryover into the sperm fraction. As a result, the autosomal to Y-DNA ratio of sperm fraction extracted from swab #2 indicated an overwhelming amount of female DNA, which is further evidenced by the presence of a single peak representing X amplicon at the Amelogenin locus. It should be noted that it is possible to reduce this carryover by exposing the swab to longer digestion times. Twice the amount of female DNA was present in the sperm fraction extracted from swab #3 as determined by the ratio of relative fluorescence units (RFU) in the X and Y sex typing alleles at Amelogenin locus. The autosomal to Y-DNA ratio of sperm fraction extracted from swab #1 is 0.6 and this indicates the presence of predominantly male alleles (Table 2). A clean male autosomal STR profile was obtained, which is identical to the sample extracted with the method used by Broward sheriff's office crime lab, and with much shorter processing time needed in comparison.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

## REFERENCES

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We claim:

1. A method of separating male sperm cells and female epithelial cells in a sample embedded in a cotton swab, comprising:  
immersing the sample in an alkaline solution comprising 0.2-0.8 N NaOH,